Cryptic Biodiversity among Plant Pathogens: Implications for Trade and Disease Management

Door Prof. dr P.W. Crous

Leerstoel "Evolutionary Fytopathologie"
Leerstoelgroep Fytopathologie, Kenniseenheid Plant
Wageningen, 16 september 2004

Phytomycology as science

Geachte Rector, geachte collegae en vrienden. Om U in staat te stellen deze inaugurale rede in zijn context te plaatsen moet ik eerst een wetenschapsgebied introduceren dat ik "phytomycologie" noem. Voor de phytopatholoog is het uitgangspunt het ziekteproces, veroorzaakt door schadelijke organismen zoals insecten, virussen, bacteriën en fungi. Belangrijkste doelstelling is de ziekte onder controle te krijgen en waar mogelijk uit te bannen. Mycologen zijn daarentegen systematici, die fungi beschrijven. Een "phyto-mycoloog" is dus een mycoloog die zich bezig houdt met het karakteriseren van phytopathogene schimmels. Hij vormt een brug tussen enerzijds de phytopatholoog met de zieke plant, en anderzijds de mycoloog met het schadeverwekkend organisme.

In de praktijk is phytomycologie even oud als de plantenpathologie, omdat veel van de ziekteverwekkers schimmels zijn. Aanvankelijk werd de systematiek grotendeels bepaald door de waardplant: andere soort, dan ook een andere ziekteverwekker. Men nam dus aan, dat de meeste fungi een hoge waard-specificiteit hadden. Later werden morfologisch identieke schimmels op talloze, volkomen onverwante plantensoorten aangetroffen. Die schimmels doen maar wat, was de conclusie, en als gevolg daarvan kregen enkele ziekteverwekkers soms honderden synoniemen. Recentelijk, met het op grote schaal toepassen van moleculaire technieken, waarmee vragen met betrekking tot speciatie en waardplant-specificiteit zinvol te beantwoorden zijn, ontstaat het gevoel dat de oorspronkelijke benadering er niet eens zo heel ver naast zat. De phytomycologie is bezig zich tot een zelfstandig vakgebied te ontwikkelen.

Het Centraalbureau voor Schimmelcultures (het CBS dus) heeft in mei dit jaar zijn honderdjarig bestaan gevierd met een special symposium met als titel "CBS Centenary: 100 years of Fungal Biodiversity & Ecology". Het CBS heeft zich vrijwel vanaf het begin van zijn bestaan ook bezig gehouden met phytopathologie, vooral onder leiding van Prof. dr. Johanna Westerdijk. Zij was professor aan de Universiteiten van Utrecht (1917) en Amsterdam (1930), maar had ook sterke banden met het onderzoek in Wageningen, met name met de Landbouwhogeschool en de Plantenziektenkundige Dienst. Gedurende haar loopbaan als professor heeft ze 56 studenten met succes naar de doctorstitel begeleid, waarvan er 25 vrouwen waren. Twee hiervan kwamen uit Zuid-Afrika, namelijk Margaretha Mes en Susarah Johanna Truter. Prof. dr. Mes, die wel beschouwd wordt als haar meest begaafde promovenda (Löhnis 1963), stichtte het Margaretha Mes Institute for Seed Research in Zuid-Afrika. Susarah Truter werd professor en hoofd van het departement Microbiology and Plant Pathology van de University van Natal, Pietermaritzburg. Daarnaast was zij de eerste vrouw ter wereld die decaan werd van een landbouwfaculteit. Mijn

promotoren, professor P.S. Knox-Davies en prof. M.J. Wingfield hebben beide aan de Universiteit van Natal gestudeerd, waardoor mijn aanwezigheid hier vandaag kan worden geïnterpreteerd als de voltooiing van de cirkel, de terugkeer van een verloren zoon, die na vele jaren weer op het oude nest neerstrijkt.

The talk I plan to deliver today is divided into tree parts:
In part one I will be making several statements, which I will briefly discuss and illustrate with different examples from research conducted in my own group.
In part two, I will briefly comment on what I envisage with the teaching component at WU.
In part three, I will focus in my current and future research plans here in the Netherlands.

An important aspect of global trade is to provide free access to various markets. This is crucial for developing countries, where producers can earn a stable income from exporting various agricultural and forestry products. This does mean, however, that importing countries make themselves vulnerable to receiving a range of new and potentially devastating plant pathogenic fungi. Most inspections rely on a visual "disease free" inspection, and questions are often raised about whether this is sufficient, given that many of these plant pathogenic fungi seem to be imported as endophytes, or latent pathogens in apparently healthy tissue. Quarantine officers mostly deal with lists of names relating to various plant pathogenic fungi. The problem, however, is that most of these pathogens are in fact species complexes, and that such lists rarely remain at the state of the art due to constant new systematic treatments. Furthermore, a question arises about whether plant materials bearing pathogens known already to occur in the importing regions or countries should be allowed entry, as bringing in new examples of these seeming well-known pathogens frequently introduces new genetic diversity or mating types, which could be as devastating as the introduction of a completely new pathogen.

Inadvertent introductions of fungi have had dire consequences to natural environments and cultivated crops on various continents. The economic impact of such introductions can be seen in yield loss, in increased input costs for cultivation and disease control, and in social impact. One of the best known examples is *Phytophthora infestans*, and the resulting Irish potato famine, which led to the emigration of more than 3 million people. Introduced microorganisms can also have negative effects on nature and ecosystems, for instance the effect of *Cryphonectria parasitica,* which essentially exterminated the native chestnut trees of the U.S.A. and Canada.

We are presently living in a global village, where the agricultural produce from one country can be served in households in another within days of harvest. The pathways that have been set up for the global movement of agricultural and forestry products are inextricably interlinked, and will continue to be so in future. The occurrence of fungi in imported plant materials is commonly used as a reason for recommending rejection of shipments, a process that depends on the name linked to the organism.

In some cases these quarantine fungi are not even major pathogens, but their occurrence can lead to huge economic losses, as it may give political justification for certain countries to close their borders to others, resulting in shiploads of produce

being denied access. The recent debacle with karnal bunt of wheat in the U.S.A. and elsewhere (Crous *et al*. 2001) is but one such example. In such cases, a relatively minor pathogen can result in economic losses that far exceed those associated with major pathogens. If certain pathogens occur in a country, other countries could justifiably refuse to accept produce from it. The economic consequences of such decisions are devastating to the counties concerned. These decisions rely on names. Phytomycology, the science that provides the correct names for plant pathogenic fungi, strongly influences world trade and related policy.

Part one

"Show me a plant pathogen, and I will show you a species complex"

Extension plant pathologists treat disease symptoms, and rarely deal with the organism as such. In most cases (especially in developing countries), control strategies and fungicides are recommended to farmers based on visual symptoms as observed in the field. These disease symptoms are normally "typical" for the species associated with the disease. However, we now have a range of advanced molecular techniques that suggest that most if not all pathogens represent a "cloud" of closely related sibling or cryptic species. Morphological and genetic similarities suggest that these strains have evolved from a common ancestor. Given our sporadic sampling, we probably encounter the outliers from the "cloud", and given that they are relatively far removed, they contain morphological differences, and we end up recognizing them as species. Continued sampling, however, results in collections which do not fit the neat boxes we recognized in the past, but instead disappear into the cloud of closely related strains, which over time, given favourable conditions, develops into a species complex. I will briefly illustrate it with some examples below:

The genus *Mycosphaerella* contains several thousand species (Corlett 1991), which are usually assumed to be host specific. Although recent molecular data have indicated that some species exist that have wider host ranges (Crous, unpubl.), this is generally the exception, and not the rule. A crop that has been studied rather intensively these past few years is *Eucalyptus*. Eucalypt trees are commonly cultivated via cuttings for the paper and pulp industry. Most of these species are native to Australia and Papua New Guinea (Poynton 1979). The genus *Eucalyptus* includes 700 odd species (Potts & Pederick 2000), and has in excess of 60 species of *Mycosphaerella* associated with leaf spots and cankers on one or more of these species. What is especially intriguing is that many of the *Mycosphaerella* species have travelled to other continents with their hosts, probably as endophytes in asymptomatic material (Verkley *et al*., in press). *M. suberosa*, for instance, was first found causing disease in South America (Crous *et al.* 1993b), and only later in Indonesia (Crous & Wingfield 1997), and finally in Australia (Carnegie *et al*. 1997). Various eucalypt species have in the past been transplanted to other continents, and the *Mycosphaerella* populations that were taken along seem to have genetically diverged within this time. In some cases these disjunct populations have diverged enough to be recognized as distinct species.

Mycosphaerella heimii was first described from Madagascar in 1946 (Bouriquet 1946). *Eucalyptus* plantations were established on Madagascar with seed and cuttings introduced from Indonesia. Not surprisingly, therefore, later collections found this pathogen also to be well represented in Indonesia (Crous 1998). *M. heimii* is morphologically similar to two other closely related species, namely *M. heimioides*,

which also occurs in Indonesia, and *M. irregulariramosa*, which occurs in South Africa (Crous 1998). Moreover, a multigene phylogeny of isolates collected on *Eucalyptus grandis*, and *E. globulus* in Brazil and Hawaii suggests that the *M. heimii* complex has radiated widely with this crop, and that isolated populations are currently evolving into separate sibling species that are still morphologically similar, but genetically distinguishable.

The genus *Botryosphaeria* is commonly associated with stem cankers, leaf spots and fruit rots of many hosts. Species in this complex are notoriously difficult to identify, and until recently, most taxa were simply referred to as representative of the *B. dothidea*/*ribis* complex. In recollecting these species, and by employing a multigene phylogeny, Slippers *et al.* (2004a) were able to distinguish *B. ribis* and *B. dothidea*, as well as the closely related *B. parva* and *B. lutea*. Based on similarities within the ITS ribosomal gene, we initially suspected that *B. lutea* was the dominant pathogen of numerous hosts in the Southern Hemisphere. However, a multi-gene phylogeny revealed that isolates obtained from *Acacia* in Australia were in fact representative of a new species, *B. australis* (Slippers *et al*. 2004b). Further collections and research also revealed *B. lutea* and *B. australis* to be the major pathogens involved with dieback of eucalypts, grapevines and Proteaceae in Australia and South Africa. Current research findings further suggest that most of the well-established names in *Botryosphaeria* are in fact several separate species, and that detailed research would be required to resolve their identities in future. It would appear that most records of these fungi in the literature have to date been incorrect, and that they in fact represent other, recently described, or as yet undescribed species. Once again, it appears that many of these species have been introduced along with their hosts. Earlier work by Smith *et al*. (1996) has shown that these species commonly occur as endophytes in apparently healthy tissue. New introductions could thus, even if the same species is involved, enhance the genetic diversity and fitness of an existing population.

Cylindrocladium scoparium has been associated with a wide range of plant disease problems in over 30 families throughout the world (Booth & Gibson 1973, French & Menge 1978, Peerally 1991, Waipara *et al*. 1996). This species is, however, the most commonly incorrectly identified taxon in the genus (Crous 2002). *C. scoparium* is frequently confused with other species with 1-septate, small conidia. These include *C. ovatum* (ovoid vesicles), species in the *C. floridanum* complex (sphaeropedunculate vesicles) and species in the *C. candelabrum* complex (obpyriform vesicles). These confused groups appear to account for all *C. scoparium* identifications from areas outside North America and Brazil (Schoch *et al*. 1999), which are the only places where the real *C. scoparium* occurs (Crous *et al*. 1993a).

Because these taxa are morphologically very similar to one another, Schoch *et al*. (1999) employed a biological species concept, and attempted to mate all isolates. Isolates that mated were compared morphologically and molecularly. Data from the the gene region sequenced, the ITS ribosomal region, proved inadequate to distinguish taxa, and therefore an additional database of β-tubulin sequences was compiled (Schoch *et al*. 2001). In recent studies where more isolates and gene loci were examined (Wingfield *et al.*, unpubl.), it became clear that in some populations, speciation was as yet incomplete. Some groups of isolates identified based on the biological/morphological species complex they belonged to contained more than one phylogenetic species. Although these isolated populations could be recognized as

clearly different phylogenetic species, they still retained the ability to mate. This finding illustrates the importance of considering more than one species concept when examining isolates, and also shows the benefit of including an adequate number of strains and of sequencing more than one informative gene region, as not all genes evolve at the same rate.

"Intraspecific variation, e.g. clones and mating types, represents a level of complexity that is as significant as interspecific variation"

The occurrence of both mating types means that when conditions are favourable, the organism can undergo sexual recombination, which can result in a vast number of new progeny, many of them representing new lineages or clones that could become dominant over time. The most obvious example for us today concerns *Phytophthora ramorum*, which is killing all the *Rhododendron* bushes growing along the streams in the Netherlands, as well as elsewhere in Europe. Contrary to the situation in the U.S.A., one mating type of *P. ramorum* appears to be dominant in Europe (Werres & De Merlier, 2003). Given the devastation associated with *P. infestans*, and the renewed problems associated with the later introduction of its opposite mating type, there is now considerable concern about the movement of plant species known to carry *P. ramorum*. The introduction and spread of its opposite mating type would enable it to adapt more rapidly, thus further enhancing its potential as plant pathogen.

Gonthier *et al*. (2004) recently reported the introduction of a novel population of *Heterobasidium annosum* from eastern North America into the Presidential Estate of Castelporziano, near Rome (Italy). They were able to link these isolates with the corresponding US isolates thanks to a unique insertion in the mitochondrial ribosomal operon. They also were able to infer that this population was introduced into Italy on wooden transport crates shipped to the 5th US army, who occupied the estate grounds during World War II. An equally interesting case of population movement can be found with *Sphaeropsis sapinea*, which causes bluestain of pine timber and frequently causes serious damage in pine plantations damaged by fire or hail. This pathogen is a common inhabitant of pine shipping crates that are offloaded in harbours around the world, and different vegetative compatibility groups of *S. sapinea* can be shown to be easily distributed around the world in this manner (Wingfield *et al*., unpubl.).

When we described the hyphomycete genus *Phaeoacremonium* (Crous *et al*. 1996), this went past without much attention. It was only later, when this fungus was shown to be one of the primary causes of Black Goo or Petri Disease of grapevines, that headlines like the "Grapes of wrath" and the "End of the grapevine industry" started to appear in popular press. Although the same fungi also cause phaeohyphomycoses in humans, this did not receive much attention. Grapevines and wine were much more important! (If nothing else, this shows that humans know what is important in life! Our recent discovery of the *Togninia* sexual state of *Phaeoacremonium* (Mostert *et al*. 2003), again heralded an important plant pathological breakthrough, as it now showed that these organisms were recombining, and it helped explain other data showing that they were speciating at a rapid rate. Molecular analysis of populations reveal that there are many more species out there, and that these mating types are important tools in the fungal toolbox, enabling rapid evolution, which may suggest that the fate of the wine industry is still to be decided.

"Modern agricultural management practices deliver high crop yields, but also select and cultivate the super pathogens of tomorrow"

Disease management practices influence the evolution of pathogens. Speciation within a given geographical area is influenced when the ancestral population is exposed to different ecological niches (Smith 1966). Crop and disease management practices place a certain amount of evolutionary pressure on plant pathogens. If a field contains several closely related but different sibling lineages or species, human intervention and management practices could eventually determine which sibling will become dominant. It is not easy to understand all the evolutionary consequences that human involvement have for fungal pathogens. Research has shown, for instance, that grafting and pruning equipment can promote the transmission of highly virulent isolates (Ewald 1988).

Gudelj *et al*. (2004) grouped sibling species into three groups based on their host ranges, namely species that are host specific (group 1), species that have partially overlapping host ranges (group 2), and thirdly, species that have completely overlapping host ranges (group 3). One mechanism that may influence the evolution of progeny is the relationship between propagule type and mode of spore dispersal (rain, wind). On bananas, for example, *M. fijiensis* produces more ascospores (wind-dispersed), whereas *M. musicola*, which is a less important pathogen, produces more conidia (rain-dispersed) (Stover 1980). Other mechanisms, such as the seasonality of spore production, may also play a role (Gudelj *et al*. 2004).

"Today's introduction forms tomorrow's hybrid on yesterday's host"

The introduction of plant pathogens into a new environment exposes them to new biotic and abiotic influences, such as different climatic conditions, vectors, and hosts. These changes facilitate rapid evolution (Brasier 1995). Such a newly introduced pathogen will often be subject to novel or episodic selection, which may be influenced by contact between two genetically similar, but previously geographically isolated pathogens. The coming together of two such separated lineages creates the opportunity for pathogen modification via interspecific gene flow.

Dutch elm disease is caused by *Ophiostoma ulmi*, and *O. novo-ulmi* (the latter including two subspecies). These two species can interbreed, resulting in fertile progeny with reduced virulence (Brasier 1977, Kile & Brasier 1990). If *O. novo-ulmi* is introduced into an area, it quickly replaces *O. ulmi* (Brasier 1986). During this period, however, the two species are in close contact, and gene transfer can occur between them, which influences the population structure, and can have beneficial effects on the pathogen. In Oomycetes, this is also a common phenomenon. In the Netherlands, a new *Phytophthora* species that has been found on *Primula* and *Spathiphyllum* is actually a hybrid between *P. cactorum* (endemic species) and *P. nicotianae* (an introduced species) (Man in't Veld *et al*. 1998). In South Africa, Campbell *et al*. (1999) induced sexual matings between the spot and net type of *Pyrenophora teres*, which causes net blotch of barley. The resulting progeny were found to be able to infect cultivars that were usually only susceptible to the net type, in addition to those only susceptible to the spot type (Campbell & Crous 2003). Hybridisation thus results in a different genetic makeup that influences not only the virulence of the pathogen, but also its host range.

Armillaria root rot is a well-known disease on Proteaceae in different regions in the world, including Australia (Porter *et al. 19*96), California (Farr *et al.* 1989), Hawaii (Laemmlen & Bega 1974), Kenya (Denman *et al*. 2000), Madeira (Moura & Rodriques 2001), New Zealand (Pennycook 1989), Tanzania (Denman *et al*. 2000), and Zimbabwe (Masuka *et al. 19*98). Although the disease is well-known on many plant species in Africa, it has only recently been reported from indigenous Proteaceae in the Kirstenbosch Botanical Garden of South Africa (Denman *et al*. 2000). RFLP profiling of South African isolates indicated that there were two *Armillaria* species present (Coetzee *et al*. 2003). Although it was originally expected that these species would be African taxa, this was shown not to be so. The species present were *Armillaria mellea* and *A. gallica,* are both known to be native to the Northern Hemisphere; they have clearly been introduced into the gardens. Coetzee *et al*. (2001) established that *A. mellea s. str.* was introduced into the Dutch East India Company Gardens in the centre of Cape Town approximately 300 years ago. This most likely occurred with citrus plants that were brought to the area from Europe.

Armillaria mellea s. str. is restricted to the Northern Hemisphere, and its occurrence in the Company Gardens of Cape Town is the only recorded exception (Coetzee *et al.* 2001). It appears that the fungus has spread from the Company Gardens, where it sporulates profusely, to the nearby plants in the Kirstenbosch Botanical Garden. This has serious implications, as the whole fynbos biome of the Cape Peninsula is now threatened.

A phylogenetic study done by Coetzee *et al*. (2003) indicated that the second *Armillaria* species found in the gardens was *A. gallica,* a species possibly introduced with plants from Japan. These findings suggest that such introductions during the early European colonization of South Africa might have been more common than we previously expected. Although these pathogens were probably introduced several hundred years ago, they were always well confined in a specific garden, isolated by high buildings from the rest of the Cape fynbos. The recent finding that these pathogens now occur on the foot of Table Mountain, is incredibly serious, and these escape events could have devastating effects on the Cape fynbos as it is known today.

"Time is species"

As stated previously, species of *Mycosphaerella* are generally accepted as being host specific. This assumption, however, has rarely been tested experimentally, as few isolates have been available for molecular comparison. In a recent study characterizing *Mycosphaerella* species from *Acacia*, *M. mangii* was described as being morphologically similar to, but phylogenetically distinct from, the *M. heimii*/*M. konae* species complex (Crous 1998, Crous *et al*. 2004). *M. heimii* is known from *Eucalyptus* and *M. konae* from *Protea*. Several other isolates from *Acacia* also clustered close to species known from *Eucalyptus*, such as *Pseudocercospora basiramifera* and *P. paraguayensis*, suggesting that they could have shared a common ancestor.

Another species described from *Acacia* in Thailand is *M. thailandiae* (Crous *et al*., in press), which is morphologically similar to *M. colombiensis*, a leaf pathogen of *Eucalyptus* in Colombia (Crous 1998). In ITS sequence comparisons, these species cluster together, but in other genes, they are seen to be closely related, but distinct. What is extremely interesting is that *M. thailandiae* is known from *Acacia* and banana

(*Musa* sp.), both of which, along with *Eucalyptus*, are commonly planted as exotics in the tropics and sub-tropics. This suggests that *M. colombiensis* and *M. thailandiae* had a common ancestor that could infect one or two of these species but not all three, and that human propagation of exotic plants throughout the tropics has facilitated speciation on at least one additional host genus.

Mycosphaerella citri is an important foliar and fruit pathogen of *Citrus*, causing premature leaf drop, as well as reduced tree vigour, yield and fruit size (Mondal *et al*. 2003). In a recent phylogenetic study (Crous *et al*., in press), we were able to confirm the presence of this pathogen on two new hosts outside the Rutaceae, namely *Musa* and *Acacia*. From these results it would appear that these exotics are acting as catch-crops for some of the major foliar pathogens out there, and that by planting them in new regions, we are providing new host material to drive evolution. In other words, we are encouraging adaptation of some well-established pathogens to new hosts. This is an aspect that has never before been reported in *Mycosphaerella*, and holds serious implications not only for the newly planted host, but also for the original host from which it came.

The assumption that planting a new crop would not influence the established pathogen structure out there is thus incorrect: these findings suggest that established pathogens also begin to adapt to infect newly introduced hosts. Host specificity as we thought we knew it, is thus untrue. It fact, host/pathogen relationships appear to be always in flux within a dynamic system of constantly evolving new species that begin to arise on whatever we are planting at the time.

"If a sparrow flies to a cherry tree, it's a cherry tree sparrow. If the same sparrow sits in an apple tree, its an apple tree sparrow"
(quote, F.C. Deighton, specialist on cercosporoid fungi)
This classic quote of FC Deighton explains in a nutshell how plant pathologists have in the past treated the cercosporoid fungi – if two similar looking fungi occurred on two different plant hosts, then two different species names were created. With thousands of names, and hardly any cultures, it was extremely difficult to contradict this line of thought. It was only later, however, when pathologists started cross-inoculating *Cercospora* species on vegetables and getting disease, that some of these concepts were beginning to be questioned (Crous & Braun 2003). From our recent molecular data work we now know that for the large part, within the cercosporoid fungal group, Deighton was correct – these are different species, although they may appear similar to most people dealing with them! Within the fungi there are few rules, however, and to support this statement we should look at another genus where host specificity has played an important role, namely *Phomopsis*.

The genus *Phomopsis* contains more than 800 species, most of which are recorded as being plant pathogenic on stems, leaves, fruit or roots of various plant species (Uecker 1988). *Phomopsis* species concepts have mainly been based on host affinity. Recent studies have shown, however, that various species of *Phomopsis* are able to infect a wide variety of hosts (Rehner & Uecker 1994, Uddin *et al.* 1997, Mostert *et al.* 2001), and that host association is no longer sufficient for identification purposes. This effectively means that strains can only be identified to species level if advanced molecular techniques are employed. In a study of species causing Phomopsis cane and leaf spot of grapevines, Van Niekerk *et al*. (in press) identified 15 species.

Several of these taxa, however, provide evidence of host switching. For instance *P. amygdali*, a known pathogen of peaches and almonds (Farr *et al.* 1999), and *P. helianthi*, a known pathogen of sunflowers, were also found on grapevines. Several of the species, however, could not be identified to species level due to the limited molecular data presently available for this group. One of the unknown *Phomopsis* species also included isolates from roses and cranberries. In the case of *Phomopsis*, therefore, it appears that only a few species exhibit any sign of host specificity, and that all known species in this group should thus be treated with caution.

Part two

Who cares about the fungi?

After several discussions about a possible teaching component, it was decided that the aim of a new course should be for biology students in general, to introduce them to the exciting world of the fungi. The working title for the course is at this stage "From champagne to champignons: The impact of fungi on Society". Fungi have numerous beneficial effects and uses in society. Many of them, however, also have a darker side: they can be harmful or even deadly, or they can cause other strange effects, such as making male pigs transsexual. They can be consumed (mushrooms); they produce useful antibiotics, but also very toxic and hallucinogenic compounds, the best known of which is alcohol; and they can cause disastrous plant and human diseases. Higher fungi have also cultural value as they are an integral part of nature and are beloved by humans. All those aspects will be covered by lectures in this course that will also include field excursions and visits to laboratories that work with fungi.

Part three

Future research

With my arrival in the Netherlands and at CBS, I had to develop a research focus that would fit that of the CBS, as well as supplementing that of the phytopathology group at WU. Within CBS there was already an established group on *Mycosphaerella*, as was the case at WU and Plant Research International. To this end then, *Mycosphaerella* became my genus of choice. Questions addressed in the programme concern speciation, hybridisation, phylogeny, gene flow at the population level, the adaptation to agricultural crops of fungi from wild hosts, and the host-specificity of fungal pathogens.

The genus *Mycosphaerella* contains several thousand species (including the members of its 30-odd anamorph genera). Most species are distinguished primarily based on their host. As such, *Mycosphaerella* provides a species-rich environment to test hypotheses related to speciation and host specificity. Starting at CBS I was fortunate to be able to also have some continued momentum in my *Mycosphaerella* programme. My postdoc, Ewald Groenewald, is currently evaluating host specificity by studying the *Mycosphaerella* anamorph genus, *Cercospora*. Species of *Cercospora* are usually assumed to be host specific, which has led to the description of thousands of species. Of these, *Cercospora apii* represents the oldest name for a large complex of morphologically indistinguishable species. Cross-inoculation experiments and DNA sequence data reveal that this species may have a wide host range. Our current multi-gene phylogeny suggests, however, that there is no simple solution to resolving the *C. apii* species complex. Within the *C. apii* morphotype there are species-specific taxa, as well as broad host range taxa, all of which can only be distinguished on by means of their DNA sequence. No *Mycosphaerella* teleomorph has thus far been connected

to *C. apii*. The finding of such a teleomorph would greatly facilitate unravelling the mysteries of this complex, as recombination studies will enable us to clarify a number of aspects related to host specificity.

Cercospora beticola, which is part of the *C. apii* complex, is the most important pathogen of sugar beet in Europe. Although little is known about the races that exist within this population, this pathogen is well suited to be developed as a model to understand host specificity and speciation. The objectives of Marizeth Groenewald (PhD student) are to focus on host specificity and speciation within the *Cercospora apii* complex, using *C. beticola* as a model.

Mycosphaerella fijiensis causes Black Leaf Streak disease of banana. This disease has become a global epidemic in the last decade. Banana, being the fourth largest staple food crop of the world, is thus seriously threatened by a disease that may have implications like those of blight for potatoes 150 years ago. Although there are currently only a few species of *Mycosphaerella* known from banana, the research of Mehdi Arzanlou (PhD student) has shown that up to 13 species could be involved in this disease complex. We also have the first molecular proof that some of these species also occur on crops other than banana.

In the second phase of this project a more in-depth approach will be set up to identify the mating types involved in the various *Mycosphaerella* species on banana (Edwin Abeln, Cees Waalwijk & Gert Kema), as well as to develop new detection methods, which will be useful to ensure that these pathogens are not introduced into newly planted areas.

The *Mycosphaerella* group has strong, well-established international links, namely to the *Mycosphaerella* genomics consortium (with CIRAD, INIPAB, etc.), as well as several other *Mycosphaerella* groups working on other host plants (e.g. *Citrus*, *Eucalyptus, Phaseolus*) elsewhere. These research associations provide exciting possibilities to train students, and to further our understanding of the complex associations that exist between these pathogens and their hosts

Acknowledgements
Acknowledgements are easy to write for scientific papers, but rather difficult when it gets personal. I think that I would omit not thanking my parents for their continued support, especially in giving me the opportunity to follow my dream and study forestry, and later plant pathology. I also want to thank my professors, Peter S. Knox-Davies, who guided me through my M.Sc., and Prof. M.J. Wingfield, my PhD promoter, who to this day remains a close friend and colleague. At Wageningen University I must direct a warm word of thanks to Pierre de Wit, who really helped a lot in getting me started, and who still provides constant support. The same warm thanks extends to the *Mycosphaerella* working group under Gert Kema, who took me under their wing and involved us in *Mycosphaerella* research projects right from the onset. The CBS staff, the Management Team, the WeCo, and my deputy director, Jos Koelman, who have helped a lot during some very trying times. Rob Samson and Joost Stalpers have also been extremely helpful to my family and I during those first few chaotic months in the Netherlands. The RNAAS, especially Wil Hoekstra, and formerly Dick Bootsma, are thanked for their support and encouragement. Richard Summerbell is also thanked for commenting on this script.

Let me mention, too, the international mycological community, who are in fact also "family". Since I have moved to Holland, I have had so much encouragement and support from throughout the world that it is really hard to single anyone out in particular. I think this in essence says something about the CBS being the world's most important culture collection or bank: mycologists and plant pathologists feel that they are involved in the institute, and this is of course a nice and positive development.

I would be committing a great crime if I do not acknowledge my main pillar of support, my wife, Karina, who always has to listen to my moans and groans about what went wrong on each particular day. Fortunately, some things also go right, and then we can again celebrate! My children, Max and Mia, who have already collected their first new fungi in the Netherlands, I thank as well for their love and support. Hopefully, after today, my daughter will stop telling her teacher that her dad works in the bicycle shop, where he looks at fungi. But then, these are my two greatest passions in life, so at least she had that right!

"Laastens nooi ek julle nou hartlik uit om my te vergesel na benede waar drankies en eetgoed gereed staan. Graag wil ek hierdie voorlesing afsluit deur in goeie Afrikaans te sê, baie dankie vir u aandag."

References

Booth C, Gibson IAS (1973). *Cylindrocladium scoparium*. *CMI Descriptions of Pathogenic Fungi and Bacteria* No. 362.

Bouriquet G (1946). Les maladies des plantes cultivées á Madagascar. *Encyclopedie Mycologique* **12**, 44–45.

Brasier CM (1977). Inheritance of pathogenicity and cultural characters in *C. ulmi*: hybridisation of protoperithecial and non-aggressive strains. *Transactions of the British Mycological Society* **68**, 45–52.

Brasier CM (1986). The population biology of Dutch elm disease: its principal features and some implications for other host-pathogen systems. *Advances in Plant Pathology* **5**, 55–118.

Brasier CM (1995). Episodic selection as a force in fungal microevolution with special reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany* **73**, 1213–1221.

Campbell GF, Crous PW (2003). Genetic stability of net x spot hybrid progeny of the barley pathogen *Pyrenophora teres*. *Australasian Journal of Plant Pathology* **32**, 283–287.

Campbell GF, Crous PW, Lucas JA (1999). *Pyrenophora teres* f. *maculata*, the cause of Pyrenophora leaf spot of barley in South Africa. *Mycological Research* **103**, 257–267.

Carnegie AJ, Keane PJ, Podger FD (1997). First records of three species of *Mycosphaerella* on *Eucalyptus* in Western Australia. *Australasian Plant Pathology* **26**, 71–77.

Coetzee MPA, Wingfield BD, Harrington TC, Steimel J, Coutinho TA, Wingfield MJ (2001). The root fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* **10**, 387–936.

Coetzee MPA, Wingfield BD, Roux J, Crous PW, Denman S, Wingfield MJ (2003) Discovery of two northern hemisphere *Armillaria* species on Proteaceae in South Africa. *Plant Pathology* **52**, 604–612.

Corlett M (1991). An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. *Mycologia Memoir* **18**, 1–328.

Crous PW (1998). *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memoir* **21**, 1–170.

Crous PW (2002). Taxonomy and pathology of *Cylindrocladium* (*Calonectria*) and allied genera. APS Press, Minnesota, USA.

Crous PW, Alfenas AC, Wingfield MJ (1993a). *Calonectria scoparia* and *Calonectria morganii* sp. nov., and variation among isolates of their *Cylindrocladium* anamorphs. *Mycological Research* **97**, 701–708.

Crous PW, Braun U (2003). *Mycosphaerella* and its anamorphs. 1. Names published in *Cercospora* and *Passalora*. *CBS Biodiversity Series* **1**: 1–571.

Crous PW, Denman S, Taylor JE, Swart L, Palm ME (2004). Cultivation and diseases of Proteaceae: *Leucadendron*, *Leucospermum* and *Protea*. *CBS Biodiversity Series* **2**: 1–228.

Crous PW, Ferreira FA, Alfenas A, Wingfield MJ (1993b). *Mycosphaerella suberosa* associated with corky leaf spots on *Eucalyptus* in Brazil. *Mycologia* **85**, 705–710.

Crous PW, Gams W, Wingfield MJ, Van Wyk PS (1996). *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia* **88**, 786–796.

Crous PW, Groenewald JZ, Pongpanich K, Himaman W, Arzanlou M, Wingfield MJ (2004). Cryptic speciation and host specificity among *Mycosphaerella* spp. occurring on Australian *Acacia* species grown as exotics in the tropics. *Studies in Mycology* **50**, in press.

Crous PW, Van Jaarsveld AB, Castlebury LA, Carris LM, Frederick RD, Pretorius ZA (2001). Karnal bunt of wheat newly reported from the African continent. *Plant Disease* **85**, 561.

Crous PW, Wingfield MJ (1997). New species of *Mycosphaerella* occurring on *Eucalyptus* leaves in Indonesia and Africa. *Canadian Journal of Botany* **75**, 781–790.

Denman S, Coetzee MPA, Wingfield BD, Wingfield MJ, Crous PW (2000). Armillaria root rot. A new disease of cut-flower proteas in South Africa. *Fynbos Research* **33**, 1–6.

Ewald PW (1988). Cultural vectors, virulence, and the emergence of evolutionary epidemiology. *Oxford Surveys in Evolutionary Biology* **5**, 215–245.

Farr DF, Bills GF, Chamuris GP, Rossman A (1989). *Fungi on plants and plant products in the United States.* APS Press, Minnesota, USA.

Farr DF, Castelbury LA, Pardo-Schultheiss RA (1999). *Phomopsis amygdali* causes peach shoot blight of cultivated peach trees in the southeastern United States. *Mycologia* **9**, 1008–1015.

French DW, Menge JA (1978). Survival of *Cylindrocladium floridanum* in naturally and artificially infested forest tree nurseries. *Plant Disease Reporter* **62**, 806–810.

Gonthier P, Warner R, Nicolotti G, Mazzaglia A, Garbelotto MM (2004). Pathogen introduction as a collateral effect of military activity. *Mycological Research* **108**, 468–470.

Gudelj I, Fitt BDL, van den Bosch F (2004). Evolution of sibling fungal plant pathogens in relation to host specialization. *Phytopathology* **94**, 789–795.

Kile GA, Brasier CM (1990). Inheritance and inter-relationship of fitness characters in progeny of an aggressive x non-aggressive cross of *Ophiostoma ulmi*. *Mycological Research* **94**, 514–522.

Laemmlen F, Bega RV (1974). Hosts of *Armillaria mellea* in Hawaii. *Plant Disease Reporter* **58**, 102–103.

Löhnis MP (1963). Johanna Westerdijk een markante persoonlijkheid. H. Veenman en Zonen N.V., Wageningen, the Netherlands.

Man in't Veldt WA, Veenbaas-Rijks WJ, Ilieva E, de Cock AWAM, Bonants PJM, Pieters R (1998). Natural hybrids of *Phytophthora nicotianae* and *P. cactorum* demonstrated by isozyme analysis and random amplified polymorphic DNA. *Phytopathology* **88**, 922–929.

Maynard Smith J (1966). Sympatric speciation. *American Naturalist* **100**, 637–650.

Masuka J, Cole DL, Mguni C (1998). *List of plant diseases in Zimbabwe*. Department of Research Specialist Services, Ministry of Lands and Agriculture, Harare, Zimbabwe.

Mondal SN, Gottwald TR, Timmer LW (2003). Environmental factors affecting the release and dispersal of ascospores of *Mycosphaerella citri*. *Phytopathology* **93**, 1031–1036.

Mostert L, Crous PW, Groenewald JZ, Gams W, Summerbell RC (2003). *Togninia* (Calosphaeriales) is confirmed as teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility, and DNA phylogeny. *Mycologia* **95**, 646–659.

Mostert L, Crous PW, Kang C-J, Phillips AJL (2001). Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. *Mycologia* **93**, 145–166.

Moura MF, Rodriques PF (2001). Fungal diseases on proteas identified in Madeira Island. *Acta Horticulturae* **545**, 265–698.

Peerally A (1991). The classification and phytopathology of *Cylindrocladium* species. *Mycotaxon* **40**, 323–366.

Pennycook SR (1989). *Plant diseases recorded in New Zealand. Volume 1*. Plant Disease Division, D.I.S.R., Auckland, New Zealand.

Porter I, Pascoe I, de Boer D, Ziehrl A (1996). Major diseases of wild flowers. In *Proceedings of workshop Growing wildflowers for profit, 1995*. Cass A, Slater T & Tregea W, eds. pp. 23–39. Institute for Horticultural Development, Victoria, Australia.

Potts BM, Pederick LA (2000). Morphology, phylogeny, origin, distribution and genetic diversity of the eucalypts. In *Diseases and Pathogens of Eucalypts*. Keane PJ, Kile GA, Podger FD, Brown BN, eds. pp. 11-34. CSIRO Publishing, Collingwood, Victoria.

Poynton RJ (1979). *Tree Planting in Southern Africa. Vol. 2 The Eucalypts*. Report to the Southern African Regional Commission for the Conservation and Utilization of the Soil (SARCCUS). Department of Forestry, Republic of South Africa.

Rehner SA, Uecker FA (1994). Nuclear ribosomal internal transcibed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. *Canadian Journal of Botany* **72**, 1666–1674.

Schoch CL, Crous PW, Wingfield BD, Wingfield MJ (1999). The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* **91**, 286–298.

Schoch CL, Crous PW, Wingfield BD, Wingfield MJ (2001). Phylogeny of *Calonectria* based on β-tubulin DNA sequence comparisons. *Mycological Research* **105**, 1045–1052.

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ (2004a). Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **96**, 83–101.

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2004b). Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* **96**, 1028–1039.

Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996). *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**, 86–88.

Stover RH (1980). Sigatoka leaf spots on bananas and plantains. *Plant Disease* **64**, 750–756.

Uddin W, Stevenson KL, Pardo-Schultheis RA (1997). Pathogenicity of a species of *Phomopsis* causing a shoot blight on peach in Georgia and evaluation of possible infection courts. *Plant Disease* **81**, 983–989.

Uecker FA (1988). A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. *Mycologia Memoirs* **13**, 1–231.

Van Niekerk JM, Groenewald JZ, Farr DF, Fourie PH, Halleen F, Crous PW (2004). Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology*, in press.

Verkley GJM, Crous PW, Groenewald JZ, Braun U, Aptroot A (2004). *Mycosphaerella punctiformis* revisited: morphology, phylogeny, and epitypification of the type species of the genus *Mycosphaerella* (Dothideales, Ascomycota). *Mycological Research*, in press.

Waipara NW, Di Menna ME, Cole ALJ, Skipp RA (1996). Pathogenicity of *Cylindrocladium scoparium* to pasture clover and grass species. *Australasian Plant Pathology* **25**, 205–211.

Werres S, Merlier D De (2003). First detection of *Phytophthora ramorum* mating type A2 in Europe. *Plant Disease* **87**, 1266.

Odd hidden note for true phytomycologists: the title of Westerdijk's inaugural lecture at Utrecht (1917) was " Der nieuwe wegen van het phytopathologisch onderzoek", and in Amsterdam (1930) "De groei der Phytopathologie". These topics remain relevant to this day, as does her legacy.